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## (54) CULTURE MEDIUM AND USE OF THE SAME

(57)The present invention relates to a medium for culture, containing human serum albumin (rHSA) obtained by gene manipulation, a method for culturing an animal cell, including culturing the animal cell in the above-mentioned medium for culture, and a method for producing a physiologically active substance, including culturing an animal cell capable of producing a physiologically active substance in the above-mentioned medium for culture to produce the physiologically active substance and harvesting the physiologically active substance from the culture. The use of rHSA having constant quality has led to the effects of retention of cell proliferation capability and production of physiologically active substance, which are of the same level as achieved when a serum-containing medium and/or a conventional serum-free medium containing plasmaderived HSA are/is used, as well as stable quality of the medium and ensured reproducibility of the culture.



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## Technical Field State Control of the State Control March 22 2 4 7 C. O. Com Bellin, C.

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[0001] The present invention relates to a medium for culture, which contains human serum albumin obtained by gene manipulation (hereinafter also referred to as rHSA), a method for culturing animal cells using said medium and a method for producing and harvesting a physiologically active substance. Talking the se-

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#### Background: Ait is, gamuless vei tementic secrydater ei jos

[0002] With the progress of DNA recombination technique in recent years, methods for producing a physio- 15 logically active substance, such as protein, as a cellproduct have been investigated. While culture of animal cells is critical for the production of such cell product. cell culture in an industrial scale requires a great amount of a medium. โดยโทคา อกรถูงเดือธิกาญการ นี้หืออยูก 🚧

[0003] There have been conventionally used a great variety of media for growing (proliferating) animal cells, which include Dulbecco's medicined Eagle's medicin (DMEM) [Morion, H. J. J. (1970) In vitro 6, 89], F12 medium [Ham, R. O. (1965) Proc. Natl. Acad. Sci. USA 53, 288]; RPMI1640 medium [Goding, J. W. (1989) J. Immunol: Methods 39, 285, UAMA 199 (1957) 519] and the like. These media require addition of serum to the medium for the growth (proliferation) of animal cells. Typically, fetal-tovine serum, equine serum, human 30 serum and the like need to be added in a concentration of about/1-15%.cg Filter to indepthelyagratic conta-

[0004] A The use of a medium containing a serum is associated with the following problems. The control is a chrangulare so hannal ed year suley in a 25

- (1) Serum its is expensive and the culture becomes cosing 18 1 farm, Tip gains your soung ex
- (2) Serum varies between ic.s. which is disadvantageous for culture fequiring remodule 1.39 0 1039
- (3) Purification contributed in the contributed and cell product is directly and the contributed and cell product is directly and the contributed and cell product is directly and cell produc
- (4) The medium is associated in risk of providing a contamination scurce of virus and mycoplasma, अम्बर्ध गरूर हा तावार पर (६८०,८९६ के 57.80基础 68.建。 ್ವಚಿತ್ರಕ್ಷಣ - ಅಪ್ಪಡಚಿತ್ರವನ್ನು

[0005] Under the circumstances, a method for decreasing the serum concentration of a medium has been investigated However, decrease in the serum concentration leads to significant degradation of profitration capability of the center eradication of the cell, which in turn results in a noticeable decrease in the yield of a desired cell product (e.g. physiologically active substance such as protein). Hence, decreasing concentration of the serum in a medium has been unfeasible. िकार x ( stairws 4 m e**rio** कार रहे राजा:

[0006] In view of the above, a serum-free medium wherein cells can be cultured without losing proliferation capability in the absence of serum has been drawing

much attention.

[0007] "Conventional serum-free media contain a plasma-derived human serum albumin (hereinafter also referred to as HSA) as an additive for maintaining proliferation capability of the cell. The plasma-derived HSA does not necessarily has constant quality. For example, the level of contaminant plasma-derived components, such as lipoprotein, protease inhibitor (e.g., a1-antitrypsin and the like), carrier protein of metal or heme (e.g., transferrin, ceruloplasmin, haptoglobin and the like), fatty acid, calcium ion, tryptophan, cysteine, glutathione, trace amount of metal component and the like, varies from one product lot to another. Thus, there is a concern that these components may expert influence on the culture of animal cells and the like.

### Disclosure of the Invention

[0008] The present invention has been made in view of the above-mentioned problems, and aims at providing a medium for culture, which has stable quality, exerts less influence on the culture, and in which cells can proliferate sufficiently.

[0009] Another object of the present invention is to provide a method for providing stable (having superior

reproducibility) culture of animal cells.

[0010] A yet another object the present invention is to provide a method for producing a physiologically active substance in large amounts from animal cells capable of producing the physiologically active substance and harvesting the same.

[0011] The present inventors have conducted intensive studies in consideration of the above mentioned situation and found that the use of an HSA (rHSA) obtained by gene manipulation as an additive for the basal medium leads to retention of sufficient proliferation capability of the cell while maintaining the effect during culture as achieved by the use of a plasmaderived HSA, which resulted in the completion of the present invention. In other words, the use of rHSA having constant quality for the medium of the present invention beneficially results in less influence (i.e., ensuring reproducibility) on the effect of culture.

[0012] Accordingly, the present invention provides the following.

- (1) A medium for culture, containing a human serum albumin obtained by gene manipulation.
- (2) The medium for culture of the above-mentioned (1), containing, as a basal medium, DMEM medium, F12 medium or RPMI1640 medium.
- (3) The medium for culture of the above-mentioned
- (1), wherein the human serum albumin obtained by gene manipulation is the albumin produced by a human serum albumin-producing yeast. ...
- (4) The medium for culture of the above-mentioned
- (1), further containing insulin.
- (5) The medium for culture of the above-mentioned

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- further containing peptone.
- (6) The medium for culture of the above-mentioned
- (1), further containing transferrin.
- mertice district (7) The medium for culture of the above-mentioned
- (1), further containing insulin, peptone and transf rrin.
- (8) A method for culturing an animal cell, comprising culturing the animal cell in the medium of the above-mentioned (1).
- (9) The method of the above-mentioned (8), wherein the animal cell itself is capable of producing a physiologically active substance or producing a heterologous physiologically active substance by transformation by genetic engineering.
- wherein the animal cell is a member selected from the group consisting of human kidney cell line, hybridoma, leukocyte, Namalwa cell, BALL-1 cell, fibroblast, lymphocyte, human kidney cell, was cell, BHK cell, COS-7 cell, MDCK cell, C127 cell, HKG cell, mouse myeloma cell, human lymphoblast cell, a parent cell for preparing human-human hybridoma, dhfr deficient cell lines thereof, HGPRT deficient cell lines thereof and ouabain resistant cell. lines thereof.
- (11) A method for producing a physiologically active substance, comprising culturing an animal cell capable of producing the physiologically active substance in the medium of the above-mentioned (1) to produce the physiologically active substance, and harvesting the physiologically active substance from the culture.
- (12) The method of the above-mentioned (11), wherein the animal cell itself is capable of producing a physiologically active substance or producing. a heterologous physiologically active substance by transformation by genetic engineering.
- (13) The method of the above-mentioned (11), wherein the physiologically active substance is a member selected from the group consisting of tissue prourokinase, urokinase, antithrombin-III, plasminogen activator, hepatitis B virus surface antigen, hepatitis pre S-B virus surface antigen, interferon, colony formation stimulating factor and monoclonal antibody.

[0013] The medium for culture of the present invention is subject to no particular limitation as long as it contains a human serum albumin (rHSA) obtained by gene ,50 manipulation. Specific examples include a basal medium supplemented with rHSA

[0014] In the present invention, the basal medium is a basic medium which is typically used and contains a carbon source generally assimilable for animal cells, and nitrogen source, inorganic salt and the like that are digestable for animal cells. Where necessary, a slight amount of effective substance may be added, such as-

micronutrient, precursor substance and the like. The basic medium may be any known medium for cell culture, such as the above-mentioned DMEM medium, F12 medium and RPMI1640 medium, which is particularly preferably RPMI1640 medium.

[0015]. The rHSA to be contained in the medium of the present invention is subject to no particular limitation as an aclong as it is a human serum albumin produced by an (1000) HSA-producing host prepared by gene manipulation: with Preferred is one substantially free of contaminant components (e.g., protein) derived from a production host. more preferably one obtained by culturing an rHSA-producing host by a known means, and harvesting and purifying from culture, filtrate, microorganism or cell by (10) The method of the above-mentioned (8), 45 known separation means and purification means and purification means. [0016] Specific examples include the following methodes and the second states the second widers are also that the

[0017] The host for obtaining the rHSA to be used in at a the present invention is subject to no particular limitation in the melanoma cell, Vero cell, Hela cell, CHO cell, WI 38 as long as it is prepared by gene manipulation. It may be disclosed in known publications or one to be developed [1786] from now. Examples thereof include microorganisms is made rHSA-productive by gene manipulation (e.g., E. coli, yeast, B. subtilis and the like), animal cell, and the like. Particularly, the host is a yeast, preferably the genus Saccharemyces (e.g., Saccharemyces S cerevisiae) or the genus Pichia (e.g., Pichia pastoris). An auxotrophic strain or antibiotic sensitive strain may a be used. More preferably. Saccharomyces cerevisiae ....... AH 22 strain (a, his 4, leu 2, can 1) or Pichia pastoris GTS 115 strain (his 4) is used as ad by seem and a Vebac manage

[0018] A method for preparing these rHSA-producing.... hosts, a method for producing rHSA by culturing the POSE host and a method for separating and harvesting the rHSA from culture may be known or analogous to a known method. For example anytHSA-producing host can be prepared by using a typical HSA gene [Japanese Patent Unexamined Publication Nos. 58-56684 (...) (corresponding to En-A-726/3), 58,90315 (corresponding 150517-(corresponding to EP- (c) ing to EP-A-79739) 'el HSA gene [Japanese Pat- :: A-91527)], by using ent Unexamined Pub. 23/00/14/20 62-29985, Japanese 🚁 Patent Unexamined Rublication No. 1-98486 (both, corresponding to EP-A-206733)), by using a synthetic sig-[Japanese Patent Unexamined sequence nal Publication No. 1-240191 (corresponding to US Patent 7.3%) No. 5409815 and EP-A-329127)], by using a serum albumin signal sequence [Japanese Patent Unexamina need ined Publication No. 2-167095 (corresponding to EP-A-319641)], by integrating a recombinant plasmid on a. Observe chromosome [Japanese Patent Unexamined Publica : 163.44 tion No. 3-72889 (corresponding to EP:A-399455)], by \_ blow fusing hosts [Japanese Patent Unexamined Rublication 2 : 1/8 ::-No. 3-53877 (corresponding to EP-A-409156)], by causing mutation in a methanol-containing medium, by using a mutant AOX2 promoter [Japanese Patent Unexamined Publication Nos. 6-90768, 4-299984 (both corresponding to ER-A-506040)] by expressing HSA by B. A. S. A. S.

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subtilis [Japanese Patent Unexamined Publication No. (corresponding to EP-A-229712)], by expressing HSA by yeast [Japanese Patent Unexamined Publication Nos. 60-41487 (corresponding to EP-A-123544), 63-39576 (corresponding to EP-A-248657), 63-74493. (corresponding to EP-A-251744)], or by expressing HSA by Pichia yeast Japanese Patent Unexamined Publication No. 2-104290 (corresponding to EP-A-344459)]. Fresh and and with mith of A.A. ii.

[0019] Of these, the method causing mutation in a 170 methanol-containing medium, includes the following of steps. That is, a plasmid having a transcription unit8. which expresses HSA under the control of an AOX1 promoter is introduced into the AOX1 gene region of a suit- mo. H abl host by a conventional method, preferably Pichia 15 yeast, specifically GTS 115 strain (NRRL deposit No. Y-3) 15851), to give a transformant [see Japanese Patent - 100 necessary. 186 . 1 Unexamined Publication No. 2-104290 (corresponding [0025] Insulin is not particularly limited as to its derito EP-A-344459)]. This transformant has weak proliference in ation capability in a methanol medium. Therefore, this 20° human insulin'is preferably used. Peptone is not partictransformant is equitured; in a limethanol-containing co. medium to-cause-mutations and conly proliferable cells in 2 o are recovered. The methanol concentration here is, for any example, about 0.0001-5%. The medium may be an it a artificial medium or natural medium. The culture condi- 25 tions are 15-40°C, about 1-1000 hours are 15-40°C, about 1-1000 hours are

[0020] The rHSA production host is cultured by a method disclosed in the above-mentioned publications, § § a method wherein high concentration cells and product \* are obtained by fed batch culture (cemi-batch culture) (~ 30 by supplying high concentration glucose or methanol in suitable small amounts while avoiding high concentration is tion substrate inhibitions of productions cells (Japanese 🚧 Patent Unexaminad Publication No. 3-83595); a method a rHSA production [lac...neso Patenti-Unexamined Publication No. 4-293495 (corresponding to US Patent No. 400) 5334512 and ER:A-5048 ...); ( ) of the like ( ) is 1000

[0021] a The rHSA produced of culture treatment is iso-101 nents derived from the host cell and culture components by various methods. For example, a conventional and agent may be those known to those of ordinary skill in method includes subjecting a yeast culture solution containing rHSA to compression—sultraffitration membrane treatment→heat treatment-substilltrations membrane 445 treatment, and further subjecting to column chromatography treatmentswith reation sexcharager, hydrophobic chromatography treatment, columnic chromatography treatment with anion exchanger and the like [Japanese 33] Patent Unexamined Publication No. 5-317079 (corresponding to US Patent No. 5440013 and EP-A-570916), Biotechnology of Blood Proteins 1993, volt 227, 293 298]. Armethod including subsequent to the abovementioned conventional method, a step of chiefate resin and treatment or a treatment of boric acid or salt thereof has been also documented [Japanese Fatent Unexamined Publication Nos. 6-56883 and 6-245789 (both corre- 11sponding to US Patent No. 5521287 and EP-A-

612761)]. 3 2 3 4 5 5

[0022]: Subsequent to heat treatment of this yeast culture solution, a stream line method using an adsorption fluidized bed technique [Japanese Patent Unexamined Publication No. 8-116985 (corresponding to EP-A-699687); and the like can be also applied. The rHSA thus prepared and purified can be formulated by a known method such as sterilization by heating, ultrafiltration membrane treatment, addition of stabilizer, sterilization by filtration, dispensing, lyophilization and the like.

[0023] The rHSA is added to the medium for culture of the present invention in a proportion of about 0.1-5 g/L, preferably 0.1-2 g/L.

[0024]. The medium for culture of the present invention may contain insulin, peptone, transferrin and the like as

vation, but a bovine derived insulin or a recombinant ularly limited as to its derivation, but beef-derived peptone is preferably used. A plant-derived peptone can be also used. Transferrin is not particularly limited as to its derivation, but human or bovine-derived one is preferably used: Mark the street was the market was

[0026] Insulin is added to the medium for culture of the present invention in a proportion of about 0.1-10 mg/L, preferably 0.1-2 mg/L. The peptone content is 0.1-50 ~ g/L, preferably 1-10 g/L, and the transferrin content is 0.5-20 mg/L, preferably 1-15 mg/L.

[0027] The medium for culture of the present invention may contain hypoxanthine (0.1-100 mg/L), preferably 1-20 mg/L, thymidine (0.01-100 mg/L), preferably 1-10 's mg/L, selenium (0.01-100 μg/L), preferably 1-10 μg/L, wherein a fatty acid is added to the medium to enhance  $\pm$  35  $\pm$  and  $\alpha$ -tocopherol (vitamin E, 0.001-10 mg/L), preferably 0.01-0.5 mg/L, where necessary.

[0028] When animal cell transformed to have a vector containing a resistance gene is to be cultured, a selection agent corresponding to the resistance gene conlated and purified at sufficient level from the composition that red in the vector may be further added to the medium to keep plasmid transformation stable. The selection the art, such as neomycin, hygromycin, micophenolic acid, hypoxanthine, xanthine, aminopterin, methotrexate (MTX) and derivatives thereof.

[0029] The animal cell that can be cultured in the medium for culture of the present invention may be capable of producing physiologically active substance by itself, or may produce a heterologous physiologically active substance by transformation by genetic engineering. The cell capable of producing physiologically active substance by itself may be, for example, hybridoma that produces monoclonal antibody, leukocyte, Namalwa cell or BALL-1 cell that produces interferon (IFN)-a, fibroblast that produces 3IFN-β, lymphocyte that produces IFN-y, human kidney cell that produces prourokinase or urokinase (proUK or UK), melanoma (e.g., Bowes cell) that produces tissue plasminogen activator

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(TPA) and the like. The established host cell to be trains (FRA) and the like. The established host cell to be trains (FRA) (F formed by genetic engineering may be, for example, Vero cell, Hela cell, CHO cell, WI38 cell, BHK cell, COS-7 cell, MDCK cell, C127 cell, HKG cell, human kidney cell line and the like. Specific examples include CHO-K<sub>1</sub> 5, JAMA 199 (1957)] was used. As additives, rHSA (1 g), (Chinese hamster ovary cell : ATCC CCL61), BHK (hamster kidney cell : ATCC CCL10), COS-7 (CV-1 Ori- 10 mg), hypoxanthine (13 mg), thymidine (4 mg), α-10 mg), gin, SV-40 cell : ATCC CRL1651), Vero cell (African tocopherol (0.13 mg) and selenium (4 µg) were used to green monkey kidney cell : ATCC CCL81), mouse myeloma cell (X63-Ag8-653; P3U1), human lymphoblast 100 from Pichia pastoris yeast, which was prepared accordcell (IM-9, ATCC CCL159), parent cell for preparing human-human hybridoma, dhfr deficient cell lines of an 116985 to not prosest a colorest to be an included again thereof, HGPRT deficient cell lines thereof, ouabain resistant cell lines thereof and the like.

[0030] An animal cell is cultured using the medium for a 15% of the Newson Subtraction Subtraction of the Newson Subtracti culture of the present invention by the following method. (1996) [0035] Human kidney cell line was adhered to micro-That is, an animal cell is cultured in the inventive and according to the medium for culture premedium placed in a culture container, such as dish, (3.5.3% pared in Example 1 to a concentration of 107 cells/miles and flask, roller bottle, spinner flask, microcarrier, microcap- Company The cells were cultured under the conditions of 37°C; sule and a culture apparatus using hollow fiber, which page 5% CO<sub>2</sub>: As a result, production of proUK correspondence of are known well for use in culture; but the container is not well ing to 0.6-2.5 U/ml was confirmed in the culture solution limited to these exemplified. The method of culture parts in 2 days. The culture solution was changed every 2-3 because the culture solution was changed every includes, for example, subculture generally performed in was a days thereafter, whereby continuous culture over a long the above-mentioned culture container, and continuous was period of time (at least one month) could be performed: culture performed for a long time under constant culture (25). (as what a smaller with marbian for the constant culture) menting the old culture solution which is continuously or with the cell or upon separation of the cell. Moreover, Other culture conditions are, for example, culture temtinuous culture can be achieved.

tein is produced by the cell by a method known in the [0037] closed in Japanese Patent Unexamined Publication No. 40 incorporated hereinto by reference and the best of the second of the sec 61-177987 (corresponding to EP-A-154272), Japanese Patent Unexamined Publication No. 63-146789 (corresponding to US Patent No. 5098840 and EP-A-253241) and the like. The sales operate pending a long to accompany to the

[0032] According to the production method of a phys- 45 iologically active substance of the present invention, for provide example, a physiologically active substance such as 2. antithrombin-III, proUK, UK, TPA, hepatitis B virus surface antigen, hepatitis pre S-B virus surface antigen, IFN, colony formation stimulating factor, monoclonal 50 antibody and the like can be produced. are anti-right planter in the growth of an endan-

# Examples

on tail beg The present invention is explained in more 55 detail in the following by way of Examples which do not ..... 4. limit the present invention in any way.

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[0034] As a basal medium, RPMI1640 medium [10.2 g, Goding, J. W (1980) J. Immunol. Methods 39, 285, insulin (1 mg), beef derived peptone (5 g), transferrin ing to Japanese Patent Unexamined Publication No. 8-14-5-7

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## the State of Figure Example 2 Culture of human kidney cell fine

ं १५ रेजीवर हो मध्ये एक स्थापन स्थित । के discontinuously extracted from the culture tank together. [0036] . The use of the medium for culture of the present invention containing human regrum albumin -the animal cell can be cultured in the inventive medium 30 (rHSA) having constant quality and obtained by gene in the form of a highly dense culture. Specifically, culture an anipulation has led to the effects of retention of cell at high density of not less than 107 cells/ml is preferable. proliferation capability and production of physiologically active substance, which are of, the same level as the perature 30-37°C, culture time 1-10 days. In addition, by special eved when a serum-containing medium and/or a " appropriately changing the medium, a prolonged con- 35 sconventional serum-free medium, containing plasma-[0031] A physiologically active substance such as pro- a summedium and ensured reproducibility of the culture. To the substance such as pro-This application is hasophor application No. 1997

field of art. Examples of the method include those dispersional and all the second of the method is 2000. alternocroup a substable blackhold occupation and browned in their

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- -record to the authorities as a second subject to the contract of the contract A medium for culture containing a human serum albumin obtained by gene manipulation: 1896 1866 1866
  - glaseau into policidos of protecejo la estracidad considera e The medium for culture of claim 1, comprising, as a  $\mathbb{R}^d \times \mathbb{R}^d$ basal medium, DMEM medium, F12 medium or an in RPMI1640, medium di bris regnistivo notae alla bi cara si
    - Cause No sumbled Publication No. 3917079 (some-The medium for culture of claim to wherein the human serum albumin obtained by gene manipula: 18.001 tion is the albumin produced by a human serum. (223) albumin-producing yeast, confirm son has a some expenses
    - Endings on the to be a product in themselves in a country. It is if The medium for culture of claim 1; further contained the c ing insuling those that exists be a degree and exist and of

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| 5.   | The medium for culture of claim 1, further containing peptone.   |
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| 6.   | The medium for culture of claim 1 further contain  |
| ٠.   | ing transferring the state of state of the s   |
| 7.   | The medium for culture of claim 1, further containing insulin, peptone and transferric.  A method for culturing an animal cell, comprising to  |
| ••   | ing insuling personal and transferring   |
|      | and a securit, peptorie and paristernic.   |
| 8.   | A method for culturing on points at the second seco   |
| ٠.   | A method for culturing an animal cell, comprising 10 culturing the animal cell in the medium of claim 1. You was a second control of the cont   |
|      | The medium of claim \$ 150 miles and the medium of claim \$ 150 miles and the state of the state o |
| 9.   | The method of claim 8 whereight a retired in the retired in the retired of claim 8 whereight a retired in the r   |
| ٠.   | The modified of cidin of Artefell file quilified Cell 1264   |
|      | is capable of producing a physiologically active sub-  |
|      | stance or producing a heterologous physiologically 15 active substance by transformation by genetic engi-  |
|      |  |
|      | the second of th   |
| 10   | The method of claim 0 when the state of the    |
| 10.  | The method of claim 8, wherein the animal cell is a  |
|      | member selected from the group consisting of 20  |
|      | human kidney cell line, hybridoma, leukocyte,  |
| •    |  |
|      | vero cell, meanona cell, vero cell, Hela   |
|      | Ceil, One ceil, vvi 38 ceil, BHK ceil, COS-7 ceil.   |
|      | MDCK cell, C127 cell, HKG cell, mouse myeloma 25 cell, human lymphoblast cell, a parent cell for organization of the collection of the cell cell for organization of the cell cell cell cell cell cell cell ce   |
|      |  |
|      | Para 9 normal rivoridoria, utili delicien cell   |
|      | lines thereof, HGPRT deficient cell lines thereof and  |
|      | ouabain resistant/cell lines thereof. 1550CT 1150 PM 1990 PM 1   |
| 44.  | Ouabain resistant cell lines thereof. 14.9 CT. White the state of the    |
|      | A method for producing a physiologically active  |
|      | substance, comprising culturing an animal cell   |
|      | capable of producing the physiologically active sub-   |
|      | the state in the s   |
|      | processing active substance, and narvesting 35   |
|      | the physiclogically active substance from the cul-   |
|      | ture.  |
| 10   |  |
| 12.  | The method of claim 11, wherein the animal cell  |
|      | itself is capable of producing a physiologically 40  |
| •    | active substance or producing a heterologous phys-   |
|      | iologically active substance by transformation by  |
| ,    | genetic engineering.   |
| 12 . | The state of the s   |
| 13.  | The method of claim 11, wherein the physiologically 45   |
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| •    | s but wound of productingse, wromsee, with the second of the second o      |
|      | The same that th   |
|      | B virus surface antigen, hepatitis re S-B virus sur-   |
| :    | ace antigen, interferon, colony formation stimulat- 50   |
| **   | ng factor and monoclonal antibody.   |
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INTERNATIONAL SEARCH REPORT PCT/JP97/02749 NO A PURPLE A. CLASSIFICATION OF SUBJECT MATTER Int. C1<sup>6</sup> C12N5/02, C12N5/06, C12N5/08, C12N5/10, C12N5/20, C12N5/24, C12P21/00, C12P21/02, C12P21/08 // C12N15/00, (C12N21/00, C12R1:91), (C12N21/02, C12R1:91), According to International Patent Classification (IPC) or to both national classification and IPC . et. 15 . e that p Lat brond sports of an 45276 4 3 FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols). Int.  $C1^6$  C12N5/00-5/24, C12P21/00-08, C12N15/00-90is above, it mines eat the mean is made to build mile in the Later . L Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched. र प्राप्त है कर कर्ण प्रताद देश अधिक है। KU # Lot Co Figure of Gag volucinems of the opening 4 1,69 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI(DIALOG), BIOSIS(DIALOG), CA(STN) end to the reference of the second to be the office of the to philipper to grown original reduction. chacker of the training withouten C. DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. Category\* p. 27-36 to without a section to the section of 竹生鸡。 Thrombosis Research, Vol. 31, No. 4, (1983), he will early be willens C. et al.,; "The Proliferation of Human Y Umbilical Vein Endothelial Cells in Serum-Free to be principled to the Medium.", see p. 623-634 a de la seculado está de desta de desta de la constante de la JP, 63-074493, A (Delta Biotechnology Ltdr) (Peology Street, S Y Fills to place with a contact and he willing & EP, 248637, A & AU, 8773786, ARA the physical option by carive substance commence as & ZA, 8703973, A & FI, 8702470, A & DK, 8702807, A & GB, 2191492, B 5 4.3 this is mine and minima. It miss to the s at deet varigational to a probability in safety नामिक राम्या विकास इस्ताविक griph के स्वाप्त का उपर र स्थापन addition are seen ्री इस्ति है किस Further documents are listed in the continuation of Box C. See patent family annex. कुल एका है है जो है तह जो क later document published after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory and orlying the invention Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance าสุโปรสรั document of particular relevance the claimed invention cannot be earlier document but published on or after the international filing date considered novel or cannot be considered to involve an inventive step when the document is these alone document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document of particular schemes; the chambel fovention calmot be. considered to involve an inventive step when the document is combined with one or more other such documents, such combination document referring to an oral disclosure, use, exhibition or other being obvious to a person shilled in the art micro sout document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family SHARE TOPY FOR Date of the actual completion of the international search Date of mailing of the international search report Decmeber 24, 1997 (24. 12. 97) November 4, 1997 (04. 11. 97) Authorized officer Name and mailing address of the ISA/ Japanese Patent Office

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